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# Randomized controlled trial of prenatal zinc supplementation and the development of fetal heart rate

**Mario Merialdi, MD, PhD,[a,b] Laura E. Caulfield, PhD,[a,*] Nelly Zavaleta, MD, MS,[c] Alberto Figueroa, MD,[c] Francesca Dominici, PhD,[d] Janet A. DiPietro, PhD[e]**

*Center for Human Nutrition, Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, Md[a]; UNDP/UNFPA/WHO/World Bank Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization, Geneva, Switzerland[b]; Instituto de Investigacion Nutricional (IIN), Lima, Peru[c]; and Departments of Biostatistics[d] and Population and Family Health Sciences,[e] Bloomberg School of Public Health, The Johns Hopkins University, Baltimore, Md*

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**KEY WORDS**
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**Objectives:** This study was undertaken to evaluate whether prenatal zinc supplementation affects maturation of fetal cardiac patterns.
**Study design:** A randomized double-blind controlled trial among 242 low-income Peruvian women was performed. Beginning at 10 to 16 weeks' gestation, women received supplements containing 60 mg iron, 250 μg folic acid with or without 25 mg zinc. Fetal heart rate (mean FHR, variability [HRV], number of accelerations) and movements (number and amplitude of movement bouts, time spent moving) were electronically monitored monthly from 20 weeks' gestation. Developmental trends were evaluated by supplement type among 195 women who completed the trial and had no serious complications of pregnancy.
**Results:** Zinc supplementation was associated with lower FHR, greater number of accelerations, and greater HRV. Supplementation effects on HRV and accelerations were more pronounced after 28 weeks' gestation. No differences in motor activity were observed.
**Conclusion:** Prenatal supplementation of zinc-deficient mothers may be beneficial to fetal neurobehavioral development.


The role of zinc in brain development and function has been progressively recognized.[1] The importance of zinc in developmental biology is related to its involvement in the expression of genetic potential, nucleic acid metabolism, and protein synthesis. Animal studies indicate that maternal zinc deficiency during the early stages of pregnancy is associated with an increased incidence of nervous system congenital malformations, whereas maternal zinc deficiency in later stages of pregnancy negatively affects neuronal growth and synaptogenesis and may be associated postnatally with impaired brain function and behavioral abnormalities.[2] In an observational cohort study in Egypt, Kirksey et al[3] reported positive

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* Reprint requests: Laura E. Caulfield, PhD, Associate Professor, Center for Human Nutrition, Bloomberg School of Public Health, The Johns Hopkins University, 615 N Wolfe St, Room W2041, Baltimore, MD 21205. Reprints supported by HD042675
*E-mail:* lcaulfie@jhsph.edu

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associations between maternal zinc status during the second trimester of pregnancy and infant development. Marginal zinc deficiency caused by inadequate dietary intake has been estimated to affect 82% of pregnant women worldwide, and thus negative effects of zinc deficiency on development would be of public health importance.[4]

Influences of maternal gestational zinc status on postnatal development would likely be expressed as subtle alterations to neurologic development in the fetus. Fetal neurobehaviors reflect neural function[5] and are continuous with postnatal functioning.[6] Recent technologic advances permit measurement of fetal neurobehavioral development by noninvasive monitoring of fetal cardiac and somatic activity. Fetal heart rate (FHR) is influenced by both neural and non-neural factors with innervation of the autonomic nervous system prominent among these. Changes over gestation in both rate and indicators of long- and short-term variability reflect the developing integration between sympathetic and parasympathetic processes.[7] During gestation, mean FHR decreases as variability and accelerations increase; both reflect increased parasympathetic control of the heart.[8,9]

Previously, we reported preliminary data indicating that maternal zinc supplementation during pregnancy was associated with greater neural control of the heart and increased fetal motor activity at 32 and 36 weeks' gestation in a Peruvian population with moderate zinc deficiency.[10] In the current investigation, we used more sophisticated recording and data analytic techniques to examine fetal neurobehavioral development in a larger sample of Peruvian women over a longer period of gestation. Because the serum zinc concentrations of women and neonates in our previous study increased yet remained low with 15 mg/d supplemental zinc,[11] we increased the dosage to 25 mg/d. We hypothesized that fetuses of zinc-supplemented mothers would develop lower mean heart rates, greater heart rate variability (HRV), more frequent heart rate accelerations, and bouts of fetal movement than fetuses of mothers not receiving supplemental zinc.

## Materials and methods

### Study design

From August 1998 to July 2000, we conducted a double-masked controlled trial of prenatal zinc supplementation among 242 Peruvian pregnant women receiving prenatal care at the Hospital Materno Infantil San Jose in Villa El Salvador, an impoverished peripheral district in Lima, Peru. In this population, maternal dietary zinc intake is approximately 8 mg/d,[12] an intake much lower than recommended intakes at that time of 15 mg/d (US Recommended Dietary Allowance). Plasma and urinary zinc concentrations are also lower than those observed in more zinc-replete populations and are responsive to zinc supplementation.[11]

### Eligibility

Women were eligible for the study if they were residents of Villa El Salvador, receiving care at Hospital Materno Infantil San Jose, considered low risk (eligible for vaginal delivery) at entry into care by medical personnel, carrying a singleton fetus, and had lived in coastal Peru for at least 6 months before becoming pregnant. Women were enrolled at 10 to 16 weeks' gestation, with duration of pregnancy at enrollment determined by last menstrual period and confirmed by ultrasonography. Women had the protocol for the study explained to them and those interested in participating signed a consent form. The Institutional Review Boards of the Instituto de Investigación Nutricional (IIN) and The Johns Hopkins Bloomberg School of Public Health approved the study protocol.

### Randomization and treatment allocation

After enrollment, women were assigned by 1 of the study physicians to 1 of 4 strata depending on parity (primipara/multipara) and week of gestation (10-13/14-16 weeks). By using computer-generated randomization lists constructed at Johns Hopkins and sent to Peru, women were randomly assigned in blocks of 2 to receive a daily supplement containing 60 mg iron (ferrous sulfate) and 250 μg folic acid, with or without 25 mg zinc (zinc sulfate). The supplements were manufactured in Lima, had the same appearance and taste, and both study personnel and study subjects were masked to treatment assignment. The randomization code was made by the pharmaceutical company and maintained in a sealed and secured envelope in IIN in Lima; it was not opened until data analyses were largely completed. Samples of the supplements were analyzed by an independent laboratory to confirm the code.

The supplements were distributed in blister packs at monthly intervals, beginning at entry into the study at 10 to 16 weeks' gestation and continuing until 1 month postpartum. Adherence with supplementation was checked biweekly by health workers who visited the women in their homes and observed the number of tablets remaining in each blister pack. The level of adherence was calculated as the percentage of tablets taken over the number of days in the study. We also queried the women by means of a standard questionnaire with specific questions regarding potential benefits or side effects of supplement consumption; no adverse side effects were recorded.

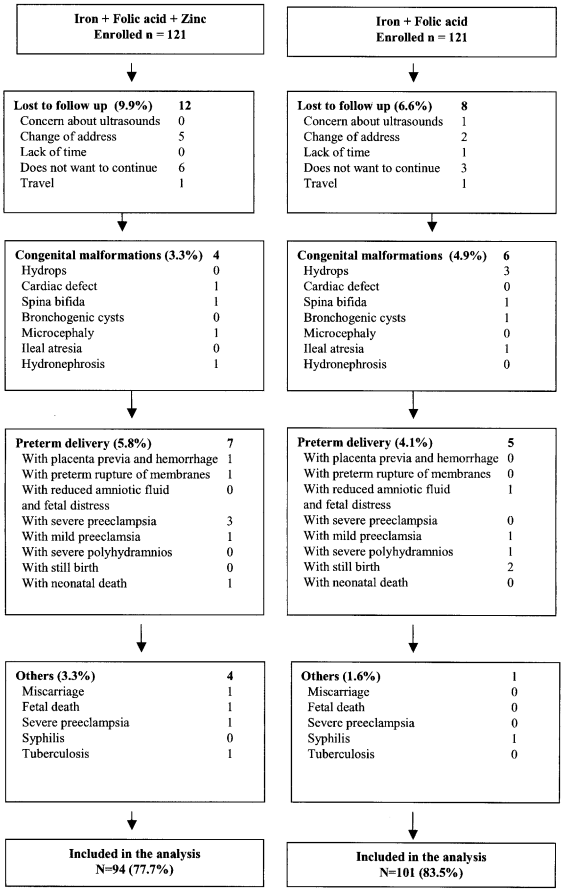


**Figure 1** Number of subjects enrolled in the study and included in the presented analysis.

## Data collection

At baseline, women were interviewed to collect sociodemographic information. At 20, 24, 28, 32, 36, and 38 weeks' gestation women came to the study clinic and underwent electronic fetal monitoring to record patterns of FHR and fetal movement, and ultrasonograhy to assess fetal growth. Maternal anthropometry (weight, height, and mid-upper arm circumference), plasma and urinary zinc, plasma ferritin, hematocrit, and hemoglobin were assessed at enrollment, 28 and 36 weeks' gestation. All deliveries occurred in hospitals. Weight was assessed at birth to the nearest 10 g by hospital personnel, and length and head circumference were assessed to the nearest 0.1 cm within 12 hours of birth by study personnel. At birth, newborn zinc concentration was determined by obtaining a sample of cord vein blood.

## Sample size

A priori sample size calculations, based on the results of our previous study,[10] indicated that a sample size of 94 women in each group would have provided a 0.8 power with a 0.05 significance level to detect differences in neurobehavioral indices by supplement type of the magnitude of 0.4 SD.

## Enrollment and follow-up

Of the 242 women enrolled in the supplementation trial, 222 (90.1%) completed the protocol and 195 (80.6%) were included in the presented analysis (Figure 1). Twenty subjects were lost to follow-up because of change of address, declination to continue participating in the study, or travel. We further excluded data from 27 subjects with significant obstetric or medical complications. The frequency of complications and congenital malformations are not unusual, and as shown, there were no significant differences in loss to follow-up or exclusions by supplement type. Exclusion from analysis for subjects who had complications develop was based on the review of medical records performed by 2 investigators (A.F. and M.M.) masked to treatment assignment and was performed before data analysis. Of the 195 women included in the analysis, 94 received zinc supplements and 101 did not.

## Outcome measures

The presented analyses include data from 1136 electronic fetal monitoring sessions performed at the scheduled gestational age ( $\pm$ 0.5 weeks). One hundred sixty-two women had 6 evaluations at scheduled times, 32 women had 5, and 1 woman had 4. Nine sessions were performed outside the scheduled gestational age interval; data from these sessions were not included in the presented analysis. Characteristics of the monitoring sessions, including time of the recording and fasting status, were comparable by supplement type.

Women were monitored for 50 minutes, while resting quietly, with a single array Doppler transducer applied to the abdomen. A fetal actocardiograph (Toitu, MT320, Wayne, Pa) simultaneously recorded FHR and movements. Data were sampled at 1000 Hz, digitized via streaming software, and analyzed offline by software (GESTATE; James Long Company, Caroga, NY) developed by 1 of the investigators (J.A.D.) to reject artifact and quantify multiple indices of fetal neurobehavioral development.[13]

The following FHR measures were quantified: mean FHR, calculated as the average of FHR during each minute of the recording; fetal HRV, calculated as the average of the standard deviations for each minute of data; and number of FHR accelerations, defined as an increases in FHR 10 beats/min above the mean FHR for at least 15 seconds.

The amplitude of the fetal movement signal produced by the actocardiograph was output in arbitrary units ranging between 0 and 100. The validity of this monitor to accurately detect ultrasound-visualized movements has been well documented, ranging from 91% to 95%

of all fetal movements whether agreement is based on time intervals or individual movements, and is equally reliable in detecting periods of quiescence.[14,15] Most movements undetected by the actograph are small, isolated movements of extremities; virtually all (97% to 98%) trunk and sustained (>1 second) movements are detected. A movement bout was defined when the actograph signal attained 15 units or greater and lasted until the signal fell below 15 units for at least 10 seconds. Tracings with few movements of high amplitude and long duration were visually inspected by 1 investigator (J.A.D.) masked to treatment assignment to assure that these unusual findings were not related to the default threshold used to detect movements. Fetal movement measures included: number, amplitude, and duration of each movement bout and time spent moving, calculated by multiplying the total number of movement bouts times the mean movement duration.

## Statistical analysis

We performed the data analysis with Stata 7.0 (Stata Corporation, College Station, Texas). To assess comparability between the 2 groups, we evaluated differences in baseline characteristics using $t$ test or $\chi^2$ analysis. We also examined the effect of zinc on fetal developmental measures separately at each gestational age, by comparing fetal developmental measures between the 2 groups using $t$ tests.

We developed a longitudinal data analysis approach to estimate associations between zinc supplementation and fetal development outcomes (treatment effects). This allowed us to take into account the correlation between repeated measurements on the same subject, and to evaluate whether treatment effects varied with gestational age. To account for the correlation among repeated observations on the same subjects, we estimated regression parameters using the generalized estimating equations method (GEE).[16] Correlation structures were chosen on the basis of the estimated autocorrelation functions of the residuals.

To estimate an overall treatment effect that does not vary with gestational age, one can fit a linear regression model of the form

$$\mathrm{E}[\mathrm{Y}_{\mathrm{iage}}] = \sum_{\mathrm{age}} \mathrm{I}_{\mathrm{age}}\beta_{0\mathrm{age}} + \beta_1 \mathrm{zinc}_{\mathrm{i}} + \varepsilon_{\mathrm{i}}$$

where $I_{age}$ are indicator variables for each gestational age, $\beta_{oage}$ are the corresponding regression coefficients, $zinc_i$ is the zinc supplementation indicator, $\beta_1$ denotes the treatment effect, and $\varepsilon_i$ are correlated errors. To evaluate whether the treatment effect varies with gestational age and to choose the appropriate modeling approach, we smoothed each fetal development outcome with respect to gestational age using lowess curves.[16] By looking at the estimated lowess curves, it appeared that at 28 weeks, HRV and number of accelerations started to differ markedly by supplement type, thus indicating that the treatment effect might vary with gestational age, before or after 28 weeks' gestation. To evaluate this possibility, we extended the previous model to:

$$\mathrm{E}[\mathrm{Y}_{\mathrm{iage}}] = \sum_{\mathrm{age}} \mathrm{I}_{\mathrm{age}}\beta_{0\mathrm{age}} + \beta_1 \mathrm{zinc}_{\mathrm{i}} + \beta_2 \mathrm{zinc}_{\mathrm{i}} \times (\mathrm{age} - 28)_+ + \varepsilon_{\mathrm{i}}$$

Under this regression approach the treatment effect of zinc ($E[Y_{iage} \mid zinc_i = 1] - E[Y_{iage} \mid zinc_i = 0]$) is a piecewise linear spline of gestational age defined as $\beta_1 + \beta_2 \times (age - 28)_+$ where $(age - 28)_+$ is equal to zero where $age < 28$ and is equal to 1 where $age \geq 28$ weeks. In this model, $\beta_1$ denotes the treatment effect before 28 weeks and $\beta_1 + \beta_2$ denotes the treatment effect after 28 weeks.

The number of heart rate accelerations and number of movements are count responses, and therefore they were also analyzed by using Poisson regression. However, exploratory analyses supported the assumption of normality, and the statistical significances of the treatment effects were not sensitive to the distributional assumptions of the outcomes (Normal distribution vs Poisson distribution). Therefore, to facilitate the interpretation of the results and their comparison across fetal outcomes, we present the linear regression results only.

**Table I** Enrollment characteristics of 195 Peruvian women by supplement type*

| | Iron + folic acid + zinc | Iron + folic acid |
|---|---|---|
| N | 94 | 101 |
| Gestation (wks) | 13.4 (1.9) | 13.4 (2.0) |
| Maternal BMI (kg/m$^2$) | 23.2 (3.1) | 23.6 (3.4) |
| Maternal height (cm) | 152.7 (5.3) | 152.3 (5.2) |
| Hemoglobin (g/L) | 121.4 (9.2) | 121.9 (10.4) |
| Plasma zinc (mmol/L) | 9.8 (1.7) | 10.2 (1.9) |
| Maternal age (y) | 23.5 (4.9) | 23.4 (5.0) |
| Parity (%) | | |
| 0 | 58.5 | 58.4 |
| 1 | 24.5 | 26.8 |
| 2 | 13.8 | 8.9 |
| >2 | 3.2 | 5.9 |
| Schooling (%) | | |
| Primary or less | 9.6 | 5.0 |
| Secondary incomplete | 28.7 | 25.7 |
| Secondary complete | 45.7 | 49.5 |
| Beyond secondary | 16.0 | 19.8 |

Presented are means (SDs) or percent.
*BMI*, Body mass index.
* No statistically significant differences by supplement type ($P > .05$).

## Results

Maternal baseline characteristics did not differ statistically by supplement type (Table I). On average, women

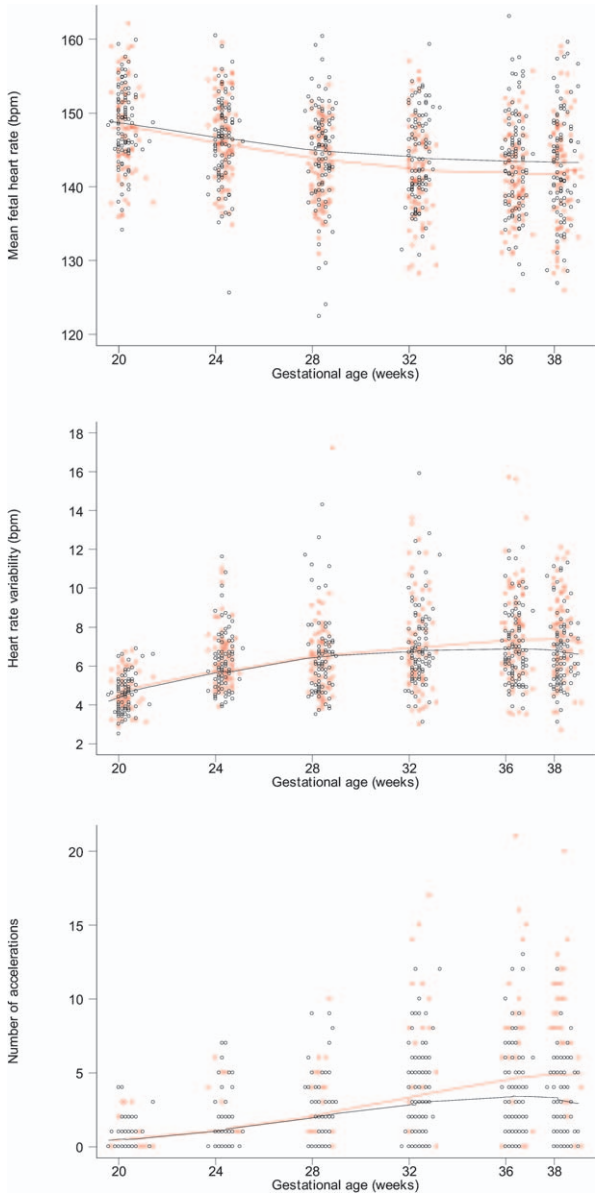


**Figure 2** Fetal heart rate measures as a function of gestational age. Lowess curve estimates of mean response in (**A**) fetal heart rate; (**B**) heart rate variability; (**C**) number of fetal heart rate accelerations by type of prenatal supplement. black line: iron + folic acid, red line: zinc + iron + folic acid. *Black circles* represent individual data points of fetuses of mothers taking prenatal supplements containing iron + folic acid, and *red circles* pertain to fetuses of mothers taking supplements that also contained zinc.

took 157 ± 27 tablets during pregnancy, with no difference by supplement type. Overall, the median level of compliance (10th, 90th percentiles), based on the number of days in the study, was 87% (71%, 97%), being 86% (68%, 97%) in the iron + folic acid + zinc group, and 88% (72%, 97%) in the iron + folic acid−only group. No statistically significant differences by supplement type were noted for duration of pregnancy, fetal sex, or birth weight (39.8 ± 1.1 vs 39.7 ± 1.0 weeks; 51% female vs 50% female; 3351 ± 427 vs 3319 ± 389 g).

Lowess curves of mean FHR as a function of gestational age by supplement type suggested that mean FHR was consistently lower in the zinc supplemented group, and that treatment effects were approximately constant over the gestational period (Figure 2, *A*). Lowess curves of mean fetal HRV and number of accelerations suggested that the outcomes become progressively greater in the iron + folic acid + zinc group after 28 weeks' gestation (Figure 2, *B* and *C*). No differences in fetal movements were observed by supplement type.

Presented in Table II are the average responses by supplement type at 28 and 38 weeks' gestation. As shown, fetuses of mothers receiving supplemental zinc exhibited lower mean FHR at both 28 and 38 weeks' gestation. At 28 weeks, there was no evidence of greater HRV and more accelerations, but differences were observed at 38 weeks' gestation. No differences were evident by supplement type in the total number of movement bouts, mean amplitude of movements, or fetal activity level.

The estimated differences in average responses (treatment effects) during gestation for fetuses of zinc-supplemented women compared with those of nonzinc supplemented women are summarized in Table III. Compared with fetuses of nonzinc-supplemented women, fetuses of women taking zinc supplements had lower mean heart rate (estimated difference: −1.13 beats/min, 95% CI −2.24 to −0.01), greater HRV (estimated difference: 0.24 beats/min, 95% CI −0.03 to 0.53), and more heart rate accelerations (estimated difference: 0.62, 95% CI 0.20-1.03). Average differences in fetal movement by supplement type were not statistically significant. Because differences in fetal HRV and number of accelerations by supplement type became more pronounced from 28 to 32 weeks onward, we tested for statistically significant differences in average responses before and after these time points using spline regression. The results indicate that differences in average responses are not significant before 28 weeks: 0.10 beats/min (95% CI −0.26 to 0.46) for HRV and 0.13 (95% CI −0.40 to 0.65) for accelerations, but are statistically significant after 28 weeks' gestation: 0.40 beats/min (95% CI 0.04-0.76) for HRV, and 1.09 (95% CI 0.57-1.62) for accelerations. In contrast, this same approach suggested that zinc supplement-associated differences in mean FHR were similar before and after 28 weeks' gestation (−1.15 95% CI −2.51 to 0.13 vs −1.10 95% CI −2.53 to 0.12).

## Comment

The results indicate that trends in FHR development are affected by maternal zinc status. We observed statistically significant differences by supplement type in the development of FHR patterns during pregnancy. In

**Table II** Comparisons in indices of fetal development in late pregnancy by type of prenatal supplement

| | 28 wks | | 38 wks | |
|---|---|---|---|---|
| | Iron + folic acid + zinc (n = 94) | Iron + folic acid (n = 99) | Iron + folic acid + zinc (n = 85) | Iron + folic acid (n = 91) |
| Heart rate (beats/min) | 142.6 (5.0)* | 144.6 (7.0) | 142.0 (7.0) | 143.2 (7.3) |
| HRV (beats/min) | 6.3 (1.8) | 6.4 (1.9) | 7.3 (2.0)* | 6.7 (1.8) |
| Number of accelerations | 1.7 (1.8) | 1.9 (2.1) | 5.0 (3.9)* | 3.1 (2.6) |
| Number of movements | 59.5 (12.8) | 59.3 (14.5) | 50.2 (14.4) | 48.5 (14.2) |
| Activity level (min) | 18.1 (7.2) | 18.1 (8.6) | 12.9 (7.8) | 13.2 (8.2) |
| Movement amplitude (arbitrary units) | 27.3 (3.7) | 26.8 (2.8) | 27.3 (3.1) | 27.1 (3.6) |

Presented are means (SDs).
* Statistically significant difference by supplement type assessed using a *t* test ($P < .05$).

a previous study[10] we observed effects of maternal zinc supplementation on fetal HRV and accelerations and on fetal motor activity. The current study confirmed our preliminary results on fetal cardiac patterns of variability and accelerations, and also demonstrated an effect of zinc supplementation on the development of mean FHR. In contrast to the prior study, we did not detect differences in measures of fetal movement by supplement type. This may be due to the use of different methods, including a higher threshold for defining movements in the previous study. If zinc affects only larger, gross body movements, a lower threshold may have obscured detection of this effect. Alternatively, it may be more difficult to consistently detect differences in fetal motor activity given the many factors that can interfere with its expression, making it a less reliable indicator of neurobehavioral development than heart rate patterns.[17,18]

The effect of zinc supplementation on heart rate became more apparent after 28 weeks' gestation. This observation may have functional implications because the gestational period between 28 and 32 weeks has been previously identified as a period of stabilization of maturation in several neurobehavioral systems, including heart rate.[13] This is also the period in gestation when the central nervous system (CNS) becomes more involved at progressively higher levels above the medulla oblongata in influencing FHR patterns. The development of heart rate patterns in anencephalic fetuses provide some of this evidence.[19-22] Mean FHR is higher in fetuses with no medulla oblongata than in fetuses in whom the medulla oblongata is conserved. HRV is conserved in anencephalic fetuses with cortex, but progressively disappears with the extension caudally of the brain defect. Accelerations are observed in anencephalic fetuses in whom the midbrain is conserved, but few are observed in fetuses with only the medulla oblongata and are absent in fetuses with no medulla oblongata.[19,21] Moreover, the brain cephalad to the medulla oblongata becomes involved in the control of FHR after 29 to 30 weeks' gestation.[22] These observations are suggestive of an upward shift in the level of brain control of FHR during this part of gestation.

**Table III** Estimated differences in average response during gestation for fetuses of zinc-supplemented women compared with those of nonzinc supplemented women

| | Difference (95% CI) |
|---|---|
| Mean FHR (beats/min) | −1.13 (−2.24 to −0.01) |
| Fetal HRV (beats/min) | 0.24 (−0.03 to 0.53) |
| Number of accelerations | 0.62 (0.20 to 1.03) |
| Number of movements | −0.36 (−2.14 to 1.41) |
| Time spent moving (min) | 0.71 (−0.55 to 2.00) |
| Movement amplitude (arbitrary units) | 0.01 (−0.43 to 0.45) |

Presented are linear regression coefficients $\beta_1$ and 95% CIs for the model: $E[Y_{iage}] = \sum_{age} I_{age}\beta_{0age} + \beta_1 zinc_i + \varepsilon_i$. To take into account of the correlation between repeated measurements, we estimated the regression coefficients using GEE with a lag1-autoregressive correlation for the first 3 variables, and with an unstructured correlation for the other variables.[16] The correlation structures were chosen on the basis of the estimated autocorrelation function of the residuals.

How zinc may influence developmental changes in neural control of FHR is not clear at present. Fetal zinc status is a function of maternal zinc status[11] and supplemental zinc ingested by the mother may have increased zinc transfer to the fetus making more available for CNS development. Zinc plays multiple critical roles in CNS functioning,[1,2] including the role of neuromodulator in the zinc-containing neurons of the cortex and limbic system (hippocampus, amygdala, hypothalamus, thalamus) and the neural circuits providing cortico-cortico, cortico-limbic, and thalamo-cortical connections. Because these areas of the brain are responsible for cognitive, behavioral, and emotional responses to environmental stimuli, our finding that zinc nutriture influences related neurobehaviors is provocative. Moreover, an emerging literature indicates that individual differences in fetal autonomic function predict aspects of autonomic functioning[23] and behavioral performance in childhood.[24,25] Thus, the results of this study may have implications for postnatal autonomic and cognitive development. Although we speculate on the mechanism through which

zinc affects fetal cardiac function, it is true that zinc has broad biologic activity, playing multiple roles in cell function and steroid hormone production and function. Thus, our results may be attributable to the effects of zinc on other modulators of cardiac function, independent of effects on overall size or maturity at birth, which were not affected by supplementation in this or our earlier studies.[10,26] Further research with animal models is needed to identify the specific mechanism(s) of action.

It is important to note that although in this study we began supplementing women with 25 mg/d zinc at 10 to 16 weeks' gestation, we previously demonstrated effects of supplemental zinc on heart rate parameters in late pregnancy when 15 mg/d supplemental zinc was initiated at 10 to 24 weeks' gestation (17 weeks on average). Although the results across the 2 studies are not directly comparable, it appears that the observed effects are not particularly sensitive to dose or timing of initiation of zinc supplementation. This may be an important consideration for those working with obstetric populations with delayed first visit for prenatal care or with access to specific formulations of zinc supplements.

In summary, the results indicate that trends in FHR development are affected by maternal zinc status. This study was conducted in a population with marginal zinc intake, similar to intakes in low socioeconomic status populations in the United States. Although our results are not strictly generalizable to other populations, they may be of broad interest to health practitioners, and certainly indicate the need for further research.

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## References

1. Frederickson CJ, Suh SW, Silva D, Frederickson CJ, Thompson RB. Importance of zinc in the central nervous system: the zinc-containing neuron. J Nutr 2000;130(Suppl):1471S-83S.
2. Golub MS, Keen CL, Gershwin ME, Hendrickx AG. Developmental zinc deficiency and behavior. J Nutr 1995;125(Suppl): 2263S-71S.
3. Kirksey A, Rahmanifar A, Wachs TD, McCabe GP, Bassily NS, Bishry Z, et al. Determinants of pregnancy outcome and newborn behavior in a semirural Egyptian population. Am J Clin Nutr 1991; 54:657-67.
4. Caulfield LE, Zavaleta N, Shankar A, Merialdi M. The potential contribution of maternal zinc supplementation to child survival programs. Am J Clin Nutr 1998;68(Suppl):499S-508S.
5. Hepper PG. Fetal behavior and neural functioning. In: Lecanuet JP, Fifer WP, Krasnegor NA, Smotherman WP, editors. Fetal development: a psychobiological perspective. Hillsdale, NJ: Lawrence Erlbaum Associates; 1995. p. 405-17.
6. Prechtl HFR. Continuity and change in early neural development. In: Prechtl HFR, editor. Continuity of neural function to prenatal to postnatal life. Philadelphia: Lippincott; 1984. p. 1-15.
7. Parer JT. Fetal heart rate. In: Creasy RK, Resnik R, editors. Maternal-fetal medicine. 4th ed. Philadelphia: WB Saunders; 1999. p. 270-99.
8. Dawes GS, Houghton CW, Redman CW, Visser GH. Pattern of the normal human fetal heart rate. BJOG 1982;89:276-84.
9. Gagnon R, Campbell K, Hunse C, Patrick J. Patterns of human fetal heart rate accelerations from 26 weeks to term. Am J Obstet Gynecol 1987;157:743-9.
10. Merialdi M, Caulfield LE, Zavaleta N, Figueroa A, DiPietro JA. Adding zinc to prenatal iron and folate supplements improves fetal neurobehavioral development. Am J Obstet Gynecol 1999;180: 483-90.
11. Caulfield LE, Zavaleta N, Figueroa A. Adding zinc to prenatal iron and folate supplements improves maternal and neonatal zinc status in a Peruvian population. Am J Clin Nutr 1999;69:1257-63.
12. Sacco LM, Caulfield LE, Zavaleta N, Retamozo L. Dietary pattern and usual nutrient intakes of Peruvian women during pregnancy. Eur J Clin Nutr 2003;57:1492-7.
13. DiPietro JA. Fetal neurobehavioral development. In: Zeskind P, Singer L, editors. Biobehavioral assessment of the newborn and young infant. New York: Guilford Publications; 2001. p. 43-80.
14. Besinger RE, Johnson TRB. Doppler recordings of fetal movement: clinical correlation with real-time ultrasound. Obstet Gynecol 1989;74:277-80.
15. DiPietro JA, Costigan KA, Pressman EK. Fetal movement detection: comparison of the Toitu actograph with ultrasound from 20 weeks gestation. J Matern Fetal Med 1999;8:237-42.
16. Diggle PJ, KungYee L, Zeger SL. Analysis of longitudinal data. Oxford: Oxford University Press; 1994 p. 45, 151-2.
17. Almi CR, Ball RH, Wheller ME. Human fetal and neonatal movement patterns: gender differences and fetal-to-neonatal continuity. Dev Psychobiol 2001;38:252-73.
18. DiPietro JA, Bornstein MH, Costigan KA, Pressman EK, Hahn CS, Painter K, et al. What does fetal movement predict about behavior during the first two years of life? Dev Psychobiol 2002;40: 358-71.
19. Terao T, Kawashima Y, Noto H, Inamoto Y, Lin TY, Sumimoto K, et al. Neurological control of fetal heart rate in 20 cases of anencephalic fetuses. Am J Obstet Gynecol 1984;149:201-8.
20. Peleg D, Goldman JA. Fetal heart patterns: a study of the anencephalic fetus. Obstet Gynecol 1979;53:530-3.
21. Kurauchi O, Ishida T, Ohno Y, Ando H, Nomura S, Mizutani S, et al. Serial fetal heart rate monitoring in monozygotic twin, one of which was anencephalic. Arch Gynecol Obstet 1995;256:53-6.
22 Yoshizato T, Koyanagi T, Takashima TSS, Akazawa K, Nakano H. The relationship between age-related heart rate changes and developing brain function: a model of anencephalic human fetuses in utero. Ear Hum Dev 1994;36:101-12.
23 DiPietro JA, Costigan KA, Pressman EK, Doussard-Roosevelt J. Antenatal origins of individual differences in heart rate. Dev Psychobiol 2000;37:221-8.
24. Bornstein M, DiPietro J, Hahn C, Painter K, Haynes O, Costigan K. Prenatal cardiac function and postnatal cognitive development: an exploratory study. Infancy 2002;3:475-94.
25. Snidman N, Kagan J, Riordan L, Shannon D. Cardiac function and behavioral reactivity during infancy. Psychophysiol 1995;32: 199-207.
26. Caulfield LE, Zavaleta N, Figueroa A, Leon Z. Maternal zinc supplementation does not affect size at birth or pregnancy duration in Peru. J Nutr 1999;129:1563-8.